Appendix (21)

ADDITIONAL PROTOCOL: HYBRIDIZATION AT LOW STRINGENCY

The detection of genes that are related but not identical in sequence to a particular probe can sometimes be accomplished by hybridizing under conditions of reduced stringency. Success depends chiefly on (1) the degree of sequence identity between the hybridization probe and the target and (2) the judicious choice of hybridization conditions. Members of a gene family from a single species or orthologous genes from different species can almost always be isolated by low-stringency hybridization if they share 65% or greater sequence identity. The identification of genes that share <65% identity requires skill in the art and sometimes luck. Genes in the latter category are more frequently isolated by low-stringency PCR (see Chapter 8). The following hybridization/washing conditions can be used to identify genes that share ≥ 65% sequence identity.

- ***For Southern hybridization or screening of bacteriophage plaques and bacterial colonies:*** Set up hybridization reactions in a buffer containing 30% (v/v) deionized formamide, 0.6 M NaCl, 0.04 M sodium phosphate (pH 7.4), 2.5 mM EDTA (pH 8.0), 1% SDS, and radiolabeled denatured probe (1×10^6 to 2×10^6 cpm/ml of hybridization solution). Hybridize for 16 hours at 42°C.
- ***For northern hybridizations:*** Hybridize in 50% deionized formamide, 0.25 M NaCl, 0.10 M sodium phosphate (pH 7.2), 2.5 mM EDTA (pH 8.0), 7% SDS, and radiolabeled denatured probe (1×10^6 to 2×10^6 cpm/ml of hybridization solution). Hybridize for 16 hours at 42°C.

At the end of the hybridization reaction, wash the membranes twice with 2x SSC/0.1% SDS for 10 minutes each at room temperature, followed by a wash for 1 hour at 55°C in 2x SSC/0.1% SDS. Use large volumes of rinse and wash solutions; make sure that they are at the appropriate temperature before use. The identification of genes that share <65% sequence is trickier but may be accomplished by using one or more of the following approaches.

- ***Use an RNA probe prepared by in vitro transcription*** (please see Chapter 9, Protocol 6). The increased stability of RNA-DNA hybrids over DNA-DNA hybrids (Casey and Davidson 1977; Zuker et al. 1985) can sometimes make the difference between seeing a signal and not seeing a signal. However, RNA probes may generate high backgrounds that are difficult to remove with low-stringency washes. The use of noncharged nylon membranes may alleviate this problem.
- ***Use a single-stranded DNA probe prepared from a bacteriophage M13 template*** as described in Chapter 9, Protocol 4 or 5. Single-stranded DNA probes generate fewer background problems than RNA probes.
- ***Decrease the formamide concentration*** to 20% and hybridize at 34°C. Rinse and wash the hybridized membranes as described above.
- ***Include "crowding agents" in the hybridization reaction.*** When included at appropriate concentrations, these agents can stabilize nucleic acids against thermal denaturation and accelerate the renaturation of DNA (for review, please see Zimmerman and Minton 1993). For a description of the effect of crowding agents on denaturation of nucleic acids, please see Wieder and Wetmur (1981) and Sikorav and Church (1991). If using DNA probes, add 10% dextran sulfate or 5% polyethylene glycol (PEG 8000) to the hybridization solutions. These polymers accelerate the rate of hybridization about tenfold (Wahl et al. 1979; Renz and Kurz 1984; Amasino 1986; Kroczek 1993). Dextran sulfate or PEG 8000 can sometimes lead to high background, and hybridization solutions containing them are always difficult to handle because of their viscosity. Use large volumes of rinse and wash solutions to overcome these problems.
- ***Use a commercial "rapid hybridization" solution*** (please see the information panels on **RAPID HYBRIDIZATION BUFFERS** and **CTAB**) and wash the membranes according to the manufacturer's instructions.